9-1-2016

Use of In Silico Structure Activity Relationship (SAR) Project in Medicinal Chemistry Course at Winona State University: Molecular Docking Simulation and Analysis of Human Dopamine D3 Receptor-Rotigotine Analog Binding

Thomas Provost
Winona State University

Myoung Lee
Winona State University

Use of *in silico* structure activity relationship (SAR) project in medicinal chemistry course at Winona State University: Molecular docking simulation and analysis of human dopamine D_3 receptor-rotigotine analog binding

Thomas Provost and Dr. Myoung Lee
Department of Chemistry, Winona State University, Winona, MN 55987

ABSTRACT

Medicinal Chemistry is a course offered by Winona State University that focuses on the chemical structure - biological activity relationship (SAR). The course is offered to chemistry and biology students who have previously taken organic chemistry and intend to further their knowledge of pharmacokinetics and pharmacodynamics. Project-based learning utilizing in silico molecular docking simulations is a primary method to help students actively learn the concept and the application of the SAR in medicinal chemistry. A drug target with a known three-dimensional structure is chosen and the amino acid residues at the binding site of the protein structure were analyzed. A lead compound was chosen and common modeling programs and lead modification strategies were employed to change the structure of the lead compound.

In this presentation, rotigotine analogs and the structure-activity relationship of analog binding to the human dopamine D3 receptor was analyzed. A single substituent was modified several times resulting in a definitive relationship of functional groups and their corresponding binding affinity. It was found that hydrophobic substituents at C1 of rotigotine increased the binding affinity while electron donating and withdrawing substituents had little effect.[1] This was due to the presence of hydrophobic amino acid residues at the binding pocket for the C1 substituent. When compared to the original rotigotine compound, it was found that replacing the original alcohol group on C1 to a hydrogen increased the binding affinity by ~300%, and by replacing the alcohol with a t-Bu an increase of ~1,800%; indicating a definitive relationship between the hydrophobicity of the functional group and the inhibition constant.[3]

BACKGROUND

Rotigotine is a commonly used anti-tremor drug that is the primary treatment of Parkinson's disease (PD) and severe restless-leg syndrome. Rotigotine belongs to a class of drugs known as dopamine agonists (DA). When rotigotine is absorbed transdermally and travels to the brain, it crosses the blood brain barrier and binds to human dopamine D3 receptor (3PBL).[2] On the specific 3PBL receptors, there are several binding sites that will be analyzed through this research using molecular docking simulation. The binding sites can be seen in figure two, where the designated binding site is circled in red. The research focus will be on the binding site of the ligand at the alcohol on carbon 1 of rotigotine, and to find out what functional groups present in the drug bind with a higher affinity to the binding site. There are multiple substituents on the rotigotine molecule that are acceptable to be analyzed. The modification site will have functional groups replacing the original substituents using a molecular modeling program. The purpose of this research is to find which functional groups offer a higher binding affinity to different amino acid residues of the 3PBL receptor that rotigotine targets. Figure one shows the sites that will be modified on the rotigotine molecule, circled in red.

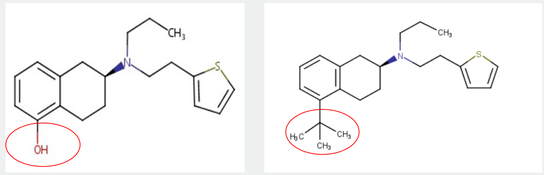


Figure 1: The structure of rotigotine along with the identification of the modification site in red. The –OH group was selected due to its location within the binding site of the protein. The second figure is the t-Bu modification that had the highest binding affinity.

OBJECTIVES

Several relationships were analyzed through the molecular docking server. The primary objectives of this experiment were to analyze:

1. Functional group interactions at binding site
2. Hydrophobicity and binding strength
3. Amino acid interactions at binding site

METHODS

Medicinal Chemistry at Winona State is a 2-crecit, upper-level, elective course. No laboratory is required and the pre-requisite for the course is organic chemistry. This course was offered in fall of 2015 with 15 chemistry and biology majors enrolled. The *in silico* SAR project was a semester-long project. Several steps were taken to conduct and analyze the structure activity relationship in the SAR project. These steps were:

1. Disease selection
2. Select a drug target with a known crystal structure/ amino acids identification in the binding site
3. Selection of the lead compound
4. Modification of the lead compound
5. Simulate docking of modified compounds/binding energy and K_I
6. Data analysis through interaction tables and binding tables
7. Assessment – oral presentation/paper/final exam

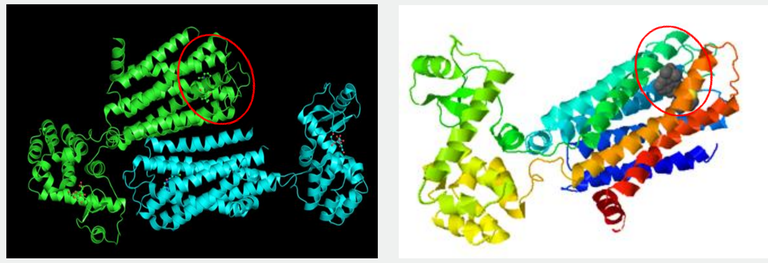


Figure 2: For the protein selection phase of the experiment, the protein 3PBL was selected. 3PBL is a two-chain, g-protein coupled receptor that binds dopamine within the human dopamine D3 receptor. It is 481 AA in length.[2] For this research, the focus was put on chain A, denoted by the color green. Figure Three: A simplified dimer of the protein with a rotigotine analogue bound to the receptor, circled in red.[3]

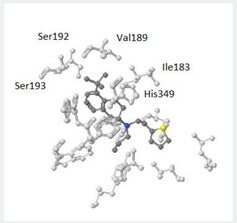


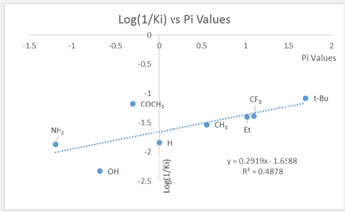
Figure 3: To further analyze the relationship of functional groups and binding affinity, the amino acid residues within the binding site were analyzed. It can be seen that Val189, Ile183, His349, Ser192, and Ser193 surround and have bonding with the carbons of the t-Bu group; the analogue that had the strongest binding.[3]

RESULTS AND DISCUSSION

Functional Group Modification	$\Delta G_{binding}$ (kcal/mol)	K_i (nM)
-OH	-9.64	212
-H	-9.76	70
$-CH_3$	-10.19	34.2
$-CH_2CH_3$	-10.36	25.5
-*t*-Bu	-10.81	12
$-CF_3$	-10.38	24.5
$-COCH_3$	-10.67	15
$-NH_2$	-9.72	74.4

Table 1: Collection of each functional group modification free energy of binding (ΔG) and inhibition constants (K_I) yielded the results above.[3] It can be seen that the strongest binding functional group was t-Bu and the original modification, OH, had the weakest binding of the investigation.[3]

RESULTS AND DISCUSSION



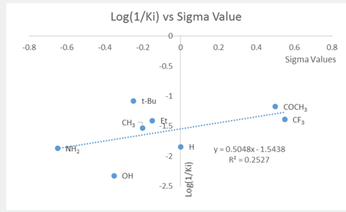


Figure 4: By taking the log($1/K_I$) and plotting each functional groups π value, a plot demonstrating the hydrophobic effect was constructed.[1] A linear relationship was identified with t-Bu being the bulkiest and hydrophobic, yet binding the tightest of all the functional groups analyzed.

Figure 5: Through the analysis of log($1/K_I$) and σ values; it was found that the electron withdrawing properties of each functional group did not have a strong effect on the binding affinity.[1] This indicates that hydrophobicity is more crucial than electron withdrawing effects in regards to binding affinity.

The experiment yielded a relationship between π values and log($1/K_I$), presenting an example of structure-activity relationship. Unlike π values, a definitive relationship between σ values and log($1/K_I$) was not present within the data. It is due to hydrophobic interactions of amino acid residues within the binding site and the selected substituent. With the presence of Val189 and Ile183 in the binding site, the K_I values for the t-Bu group are explained via the strong hydrophobic interactions and the high π value for the t-Bu group.

FUTURE PLANS

For teaching purposes, this experiment proposes a model for future medicinal chemistry students to incorporate a higher understanding of SAR. In an effort to increase the efficiency, future students will use only three ligand modifications with distinct differences in π and σ values; such as –OH, -H, -t-Bu, and $–CF_3$. A free subscription for the DockingServer is available for classroom use.

ACKNOWLEDGEMENTS

This opportunity to develop this research would not have been possible without the funding from Winona State University Student Research Grant. WSU Student Senate, WSU College of Science and Engineering, and WSU travel grants provided the funding for travel and lodging.

CONCLUSIONS

Through complete analysis of the data, it was found that the bulkier and more hydrophobic the functional group, the tighter the binding to 3PBL. This occurs because of the presence of more hydrophobic amino acid residues within the binding site. The experiment also yielded a higher understanding of SAR and an opportunity to create a future project for medicinal chemistry students.

REFERENCES

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3. Bikadi, Z.; Hazai, E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J. Cheminf.* **2009,** 1, 15.

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Hover over fill-able fields for additional guideline and completion information.

Student Name: Thomas Provost

Student Email: tprovost12@winona.edu

Student Major: ACS Biochemistry

Faculty Sponsor: Dr. Myoung Lee

Faculty Sponsor Email: mlee@winona.edu

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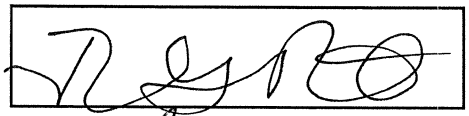
Applicant Signature: 

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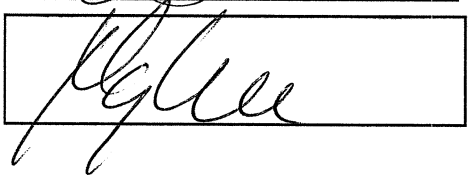
Faculty Sponsor Signature: 

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